

SCREENING FOR FREE RADICAL SCAVENGING ACTIVITY FROM ETHANOLIC EXTRACT OF MIMOSACEOUS PLANTS ENDEMIC TO THAILAND

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ABSTRACT: Preliminary study on the antioxidant activity of 20 species from the family of Mimosaceae was carried out by evaluating their free radical scavenging activity. In this study, the ethanolic extracts of pericarp, seeds, leaves and bark were allowed to react with the DPPH free radical to give a simple method but gave reliable results. Results were expressed as the concentration of the extracts necessary to inhibit the initial DPPH concentration by 50% (IC₅₀). From the IC₅₀ the free radical scavenging activities of the extracts were compared with BHT and Quercetin. Among the extracts, the *Xylia xylocarpa* extract showed the highest inhibition with IC₅₀ less than 0.1 µg/ml.

Keywords: free radical scavenging activity, Mimosaceae, DPPH, ethanolic extract

INTRODUCTION: Natural antioxidants have been receiving a lot of attention due to the uncertainty health effects of a synthetic antioxidant¹⁾. In addition to the deleterious effect of free radicals, the exploration of antioxidant sources has been promoted. Accordingly screening process has been among the earliest step taken before further study could be carried out. To date a number of plant extracts have been screening for their potential as natural antioxidant.

Free radicals are the molecules that lose one or more of their electron which make them incomplete and unstable. An antioxidant acts as a reducing agent that donates electron to the free radicals²⁾. Thus, a method to evaluate a free radical scavenging activity of plant extract have been employed by measuring the extracts ability to stabilize a synthetic free radical in a polar organic solvent such as methanol at room temperature over a period of time. For this purpose, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) synthetic radical has been commonly used. It is a stable radical with a maximum absorption at 517 nm that can readily

undergo reduction by antioxidant substances. The scavenging activity is measured from the discoloration of DPPH spectrometrically³⁾. The availability, reproducibility and convenience of this reaction have made the DPPH radical a widely used method to screen the potential of plant extract as antioxidants. As a result, it has become a routine assay in studying antioxidant.

In Thailand, the Mimosaceae plants are used as food plants, coloring material, and perfumery, in the tanning industry and as construction material. Some that very common as food are fruits of *Parkia speciosa*, *Archidendron jiringa*, *Pithecellobium dulce*, twigs of *Acacia pennata*, *Leucaena glauca*, and leaves of *Neptunia oleracea*⁴⁻⁶⁾. As food and antioxidant is closely related, this study was prompted as part of discovering the antioxidant properties of mimosaceae plants that endemic to Thailand. The aim of this study was to screen the antioxidant activity of ethanolic extracts of selected mimosaceae plant parts from their ability to scavenge a synthetic DPPH free radical.

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MATERIALS AND METHODS:

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Butylated Hydroxyl toluene (BHT) and Quercetin was purchased from Sigma Aldrich Co., Ltd. All solvents were of analytical grade.

Plant materials

Forty plant materials from 20 species of Mimosaceae consisted of bark, stem, leaves, seed coat, pericarp, stem bark, branch, twig, cotyledon, seed and branch bark were studied. Samples were collected from Ratchaburi, Rayong, Pathumthani, Nakhonpathom and Bangkok. Samples were deposited at the Faculty of Pharmaceutical Sciences Herbarium, Chulalongkorn University. The identification of each species was carried out by Associate Prof. Dr. Nijisiri Ruangrungsi. All samples were air dried before grounded and were stored at room temperature.

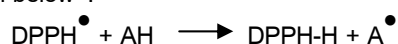
Extraction

Three different solvents namely petroleum ether, dichloromethane and ethanol were used to fractionate the soluble compound from the sample in ascending polarity. The use of sequential solvents with different polarity allows a separation of compounds according to their solubility in the solvents thus simplify and reduce the chemical complexity when taken into the extract⁷⁾.

The samples weighed 10 to 30 mg went on exhaustive extraction using a Soxhlet apparatus for 8 consecutive hours with 500 ml solvent under reflux conditions. The solvents in the extract were removed with a rotary evaporator and water bath respectively. The extracts yield were weighed, recorded and stored at -20 °C to decrease the possibility of degradation of active compound.

DPPH radical-scavenging activities

Free radical scavenging is one of the mechanisms involved in antioxidant action, a good antioxidant (AH) able to scavenge the DPPH radical and retain its own stability due to its reduction ability as shown in the equation below⁸⁾.



The scavenging activity of the extract was measured according to the method of Brand-William *et al.*³⁾ with some modifications.

Briefly, 6×10^{-5} M solution of DPPH in methanol was prepared. A 100 μl of DPPH solution was added to 100 μl of samples or standard prepared in methanol in different concentrations. Only samples from the ethanol extract were analyzed in this study. The assay was carried out in a 96 well microplate with each concentration was performed in triplicate. After 30 minutes, the absorbance of mixture was measured at 517 nm using microplate reader. A control was 100 μl of 6×10^{-5} M DPPH in methanol + 100 μl methanol. The DPPH radical scavenging activity was calculated according to the following equation:

$$\% \text{ inhibition} = (\text{A}_{\text{C}(0)} - \text{A}_{\text{A}(t)}) / (\text{A}_{\text{C}(0)} \times 100\%)$$

Where, $\text{A}_{\text{C}(0)}$ = absorbance of control at $t=0$, and $\text{A}_{\text{A}(t)}$ = absorbance of DPPH and samples at $t=30$ minutes.

A graph of % inhibition against the plant extract concentration were plotted to determine the IC_{50} , which is the concentration of the extracts necessary to inhibit 50% of the initial DPPH concentration. The IC_{50} of each extracts were compared with standards; BHT and Quercetin.

Statistical analysis

One way ANOVA was applied to compare the mean values and the significance of the differences between means was determined by Tukey's test for multiple comparisons. P values < 0.05 was regarded as significant calculated by using the Statistical Package for Social Science (SPSS) program.

RESULTS: This study was carried out to screen the ethanolic extracts from plant parts of Mimosaceae with good free radical scavenging activity from their ability to inhibit or scavenge the DPPH radical. The concentration of extract to inhibit 50% DPPH radical (IC_{50}) was shown in Table 1. From the table, extracts of bark, stem and leaves of *Xylia xylocarpa* exhibited the lowest IC_{50} values (the highest scavenging activity), which showed

that less than 0.1 µg/ml was required by those extracts to inhibit 50% of DPPH radical from their initial concentration, whereas the lowest scavenging activity was shown by the extract of *Acacia pennata* twig indicated by its high value of IC₅₀. Twenty six extracts showed IC₅₀ less than 100 µg/ml.

The IC₅₀ of extracts were compared with the IC₅₀ of both Quercetin and BHT. Quercetin is a polyphenols known for a good antioxidant activity from its structure activity relationship (SAR) whereas BHT is a commonly used phenolic synthetic antioxidant. Results from the statistical analysis showed that from the 40 extracts tested, 32 extracts showed a scavenging activity that were significantly different ($p < 0.05$) compare to BHT and Quercetin. However the ethanolic extracts of the bark, stem, leaves of *Xylia xylocarpa*, pericarp of *Archidendron jiringa* and seed coat of *Entada rheedii* showed no significant difference ($p > 0.05$) in their scavenging activity compared to Quercetin, whereas the extract from the leaves of *Acacia catechu* and *Albizia lebbeckoides* showed no difference in their

scavenging activity compared to BHT. The results were shown in figure 1.

Therefore, from the results, it was found that the ethanolic extracts from the bark, stem, leaves of *Xylia xylocarpa*, pericarp of *Archidendron jiringa*, seed coat of *Entada rheedii*, leaves of *Acacia catechu* and *Albizia lebbeckoides* were good DPPH radical scavenger that were comparable with Quercetin and BHT.

DISCUSSION: In this study, a random approach was used, where Mimosaceae plants were collected regardless of the existing studies on their chemistry or biological activity. This approach is purely serendipitous for a chance that random plant selection will give extract with good activity⁷⁾. Subsequently the plant extracts were screened using DPPH assay as this assay has been widely used, convenient and reproducible.

Mimosaceae members have been well characterized by an impressive phytochemical diversity. Polyphenols especially flavonoids and tannin are the common ones⁴⁾. Polyphenols have been known for their high

Table 1 Mean inhibition concentration (IC₅₀) of ethanolic extracts from plant parts of Mimosaceae

Species	Plant Part	Mean IC ₅₀ (µg/ml)	Species	Plant Part	Mean IC ₅₀ (µg/ml)
<i>Xylia xylocarpa</i> (Roxb.) Taub	bark	0.06	<i>Albizzia myriophylla</i> Benth.	leaves	21.93
<i>Xylia xylocarpa</i> (Roxb.) Taub	stem	0.073	<i>Leucaena glauca</i> Benth.	pericarp	37.84
<i>Xylia xylocarpa</i> (Roxb.) Taub	leaves	0.086	<i>Pithecellobium dulce</i> Benth.	leaves	52.96
Quercetin		0.45	<i>Adenanthera pavonina</i> L.	branch	58.68
<i>Entada rheedii</i> Spreng	seed coat	1.24	<i>Adenanthera microsperma</i> Teijsm.	branch	67.06
<i>Archidendron jiringa</i> I.C Nielsen	pericarp	1.38	<i>Archidendron jiringa</i> I.C Nielsen	seed	76.82
<i>Acacia catechu</i> (L.f) Willd.	leaves	2.73	<i>Leucaena glauca</i> Benth.	twig	92.02
BHT		3.47	<i>Albizia lebbeckoides</i> (D.C)	branch	109.49
<i>Albizia lebbeckoides</i> (D.C)	leaves	4.91	<i>Albizia lebbeck</i> (L.) Benth.	branch	125.21
<i>Albizia lebbeck</i> (L.) Benth.	bark	5.86	<i>Entada rheedii</i> Spreng	cotyledon	154.18
<i>Samanea saman</i> (Jacq.) Merr	leaves	6.04	<i>Neptunia oleracea</i> Lour.	leaves	186.79
<i>Entada rheedii</i> Spreng	pericarp	7.01	<i>Albizia myriophylla</i> Benth.	branch	272.68
<i>Albizia procera</i> (Roxb.) Benth.	stem bark	9.67	<i>Mimosa pudica</i> Linn.	twig	325.82
<i>Parkia speciosa</i> Hassk.	pericarp	10.03	<i>Adenanthera microsperma</i> Teijsm.	leaves	358.05
<i>Acacia catechu</i> (L.f) Willd.	branch	10.45	<i>Acacia rugata</i> Merr.	leaves	364.89
<i>Adenanthera pavonina</i> L.	pericarp	11.38	<i>Adenanthera pavonina</i> L.	leaves	442.29
<i>Cathormion umbellatum</i> (Vahl.)	leaves	13.72	<i>Cathormion umbellatum</i> (Vahl.)	branch	508.63
<i>Samanea saman</i> (Jacq.) Merr	branch	13.89	<i>Parkia speciosa</i> Hassk.	seed	569.71
<i>Cathormion umbellatum</i> (Vahl.)	bark	15.69	<i>Albizia lebbeck</i> (L.) Benth.	leaves	605.36
<i>Pithecellobium dulce</i> Benth.	stem bark	19.95	<i>Acacia rugata</i> Merr.	pericarp	937.77
<i>Acacia farnesiana</i> (Linn.) Willd.	twig	20.56	<i>Acacia pennata</i> (L.) Willd.	twig	2016.64

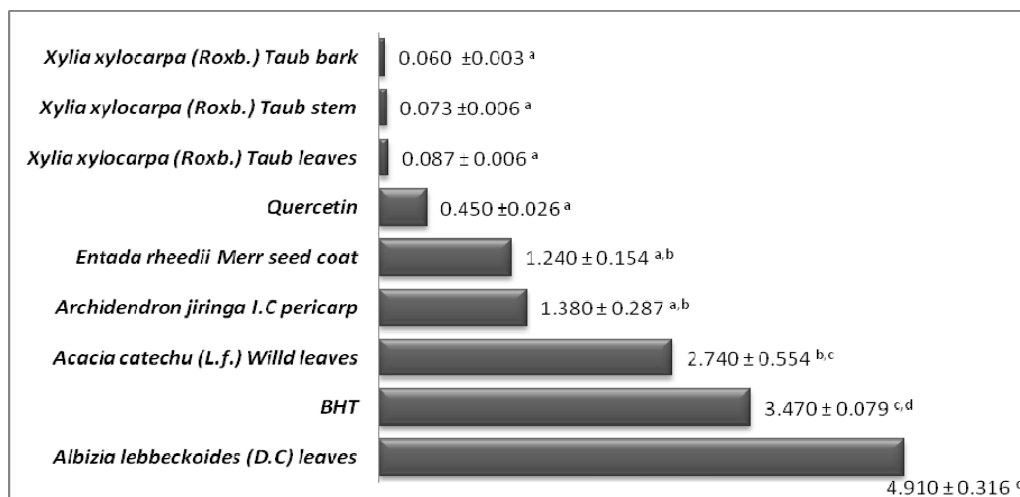


Figure 1 The comparison of the highest IC₅₀ of the extracts with Quercetin and BHT. Values are mean ± SD (n=3). The mean ± SD of the same letter are not significantly different (p < 0.05).

polarity, high solubility in polar solvents and strong antioxidant activity as well⁹⁾. Thus, polyphenols could be responsible for the free radical scavenging activity of ethanolic extract from Mimosaceae plants studied since previously research has been using ethanol to extract some high polarity compound from the biomass¹⁰⁾.

In accordance with the results is a study by Maisuthisakul *et al.*⁶⁾, that reported the bark extract of *Acacia catechu* gave the highest scavenging activity (IC₅₀ = 0.05 µg/µg DPPH), due to its high phenolic content (178mg Gallic acid equivalent/g dry weight of plant extract) and flavonoid content (42mg Rutin equivalent/g dry weight of plant extract). Ethanolic extracts from the bark, stem, and leaves of *Xylia xylocarpa* have shown a significant activity as indicated by the results. A study by Siddhuraju *et al.*¹¹⁾ reported the nutrient and chemical evaluation of raw seeds of *Xylia xylocarpa* as the cheapest and potential alternative protein source to the low income population. Our study has added information on the free radical scavenging activity of ethanolic extracts from the bark, stem, and leaves of *Xylia xylocarpa* evaluated by DPPH radical assay. Study on the extracts of *Xylia xylocarpa* will be extended to other biological activity research in the future.

Related families often contain similar type of compound and a species may yield several botanical drugs for example from the leaves and the flower. As shown by extract from bark, stem and leaves of *Xylia xylocarpa*, the value of scavenging activity were not significantly different to each other. Nevertheless, the other extract showed different activity although they are from the same species. This is due to the complexity and diversity of the chemical produced and the accumulation of chemicals are also different in the different organ of the same species⁷⁾. The synergisms of chemicals within extract also play important role in the reaction tested¹²⁾.

The chemical produced by plants are part of their defence and survivor, but no matter what are the actual reasons, they are invaluable resources that can be utilized for human benefit.

CONCLUSION: The ethanolic extracts of Mimosaceae plants were screened for their capacity to scavenge the DPPH radical. It can be concluded that most of extracts of Mimosaceae exhibited good free radical scavenging activity. Nevertheless, the ethanolic extracts from the bark, stem, leaves of *Xylia xylocarpa*, pericarp of *Archidendron jiringa*, seed coat of *Entada rheedii*, the extract from the leaves of *Acacia catechu* and *Albizia lebbbeckoides* showed very good activity that comparable with the standards. Ethanolic fractions were

known consisted mixture of polarity compounds. Thus, most probably this compounds especially polyphenols which has been recognized for strong antioxidant activities were responsible for the high free radical scavenging activity of the studied extracts. However, further separation and identification should be carried out for a better understanding. Other test for antioxidant activity will be carried out in the future to discover the full potential of Mimosaceae extracts especially extracts of *Xylia xylocarpa*.

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ฤทธิ์ต้านอนุมูลอิสระของสารสกัดเอทานอลจากพืชวงศ์กระถินในประเทศไทย

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บทคัดย่อ: ศึกษาฤทธิ์ต้านออกซิเดชันของพืช 20 ชนิด ในวงศ์ Mimosaceae โดยการศึกษาเบื้องต้นเพื่อประเมินความสามารถในการต้านอนุมูลอิสระ ใช้สารสกัดเอทานอลจากส่วนต่างๆของพืช คือ เนื้อผล เมล็ด ใบ และ เปลือกต้น ทำปฏิกิริยากับอนุมูลอิสระดีพีพีเอช วัดค่าความเข้มข้นของสารสกัดที่ยับยั้งการเกิดอนุมูลอิสระได้ร้อยละ 50 (IC₅₀) โดยใช้บีเอชทีและเคอร์เซตินเป็นสารมาตรฐานเปรียบเทียบ พบว่าสารสกัดเอทานอลของ *Xylia xylocarpa* มีฤทธิ์ต้านอนุมูลอิสระสูงสุด โดยมีค่า IC₅₀ น้อยกว่า 0.1 ไมโครกรัม/มิลลิลิตร

คำสำคัญ: ฤทธิ์ต้านอนุมูลอิสระ พืชวงศ์กระถิน ดีพีพีเอช สารสกัดเอทานอล

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